bet3–trs33–bet5–trs23, which may be linked together *in vivo* by an unknown subunit. The X-ray crystal structures of the two mammalian subcomplexes were determined; in combination with single-particle EM analysis of the yeast TRAPP I holocomplex, the structural data suggest that the particle forms an elongated, flattened, bi-lobed platform. Both the mammalian and yeast TRAPP I particles contained two copies of Bet3, which is consistent with a role for bilateral interaction between Bet3 and Sec23 on opposing vesicles during homotypic fusion of COPII vesicles in mammalian cells. One appealing model is that Bet3 independently assembles on both the vesicle and acceptor membrane, forming two distinct complexes (Bet3–Trs33–Bet5 and Bet3–Trs31–Trs20) that are bridged by Trs23 to assemble the complete tether and simultaneously stimulate GEF activity. Thus the TRAPP I holocomplex could coordinate tethering and local activation of downstream fusion machinery.

Together, these two important papers refine our model for COPII vesicle delivery to the Golgi and provide a strong foundation from which to explore the question of Rab-mediated regulation of vesicle tethering and fusion (Figure 1). Direct interaction between Sec23 on the vesicle surface and TRAPP I on the Golgi membrane (or bound through the second copy of Bet3 to another COPII vesicle) would serve to anchor the vesicle to the target membrane (Figure 1, step 1). This tethering function may be necessary to unmask the SNARE proteins, which mediate the final, short-range interaction that results in the melding of the two compartments. Binding of TRAPP I to Sec23 may also trigger guanine nucleotide exchange activity on Ypt1/Rab1 (Figure 1, step 2) that would serve to recruit the coiled-coil tether, Uso1/p115. Uso1 may further anchor the two compartments and coordinate interactions between the opposing SNAREs to further ensure specificity and maximize the efficiency of vesicle coupling with the target organelle (Figure 1, step 3) [12]. Thus, direct interaction between the tether and coat may trigger a localized molecular handoff of the vesicle-borne SNARE, released from Sec24 following Sec23–TRAPP I interaction, to the target membrane SNARE complex, coordinated by recruitment of Uso1 by activated Rab.

Interaction between a tether and a coat protein not only defines a new function for the well-characterized COPII coat, but also represents a particularly appealing model for ensuring the specificity of vesicle delivery. Indeed, coat-dependent tethering may be such an integral part of vesicle consumption that it operates redundantly in COPII vesicle transport. The putative yeast tethering complex, Grh1–Bug1, also binds Sec23–Sec24, raising the possibility that multiple tethers attract COPII vesicles to the Golgi apparatus through interaction with the coat [13,14]. More detailed characterization of coat–tether interactions and their consequences with respect to coat release, SNARE pairing and Rab activation is clearly warranted and will be aided dramatically by the availability of the crystal structure of the remarkable vesicle TRAPP.

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Plant Development: Three Steps for Stomata

Three basic helix-loop-helix proteins regulate sequential steps in the formation of stomata: SPEECHLESS initiates entry into the stomatal lineage; MUTE controls asymmetric divisions of stomatal precursor cells; and FAMA promotes guard cell differentiation.

Julie E. Gray

Stomata are microscopic pores in the aerial epidermis of plants. Each pore is surrounded by a pair of guard cells, which regulate the aperture of the pore and control gas exchange and water loss between the plant and the environment. Stomata are

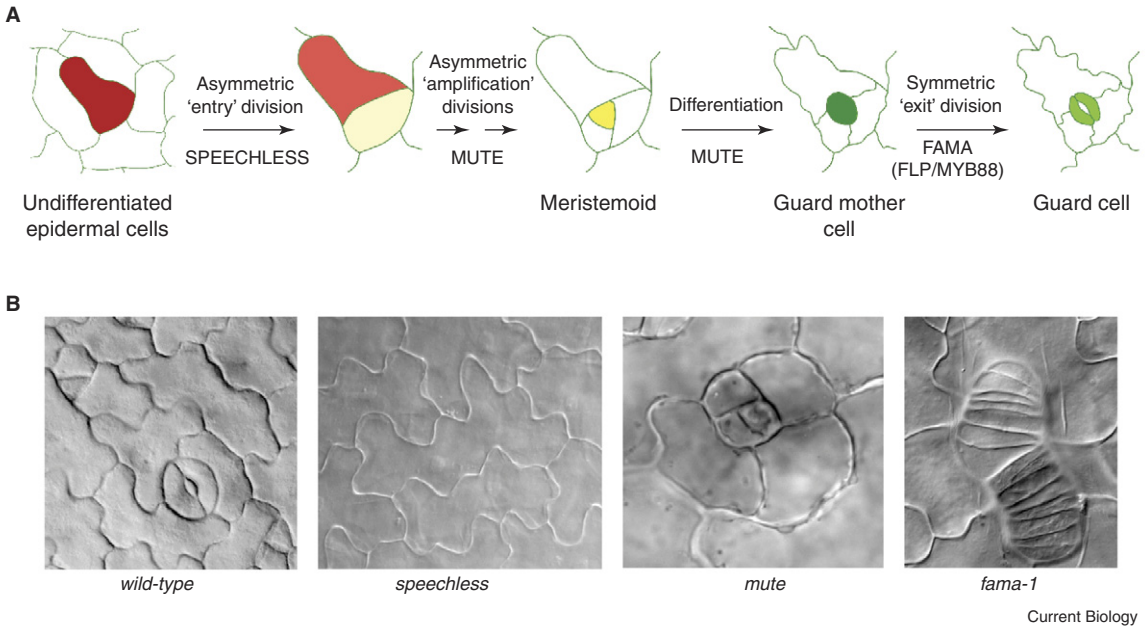

Figure 1. The stepwise development of stomata.

(A) A scheme of stomatal development illustrating the sequential expression patterns of *SPEECHLESS* (red), *MUTE* (yellow) and *FAMA* (green), and the developmental steps that they regulate in the cells of the stomatal lineage. MYB88, FOURLIPS, bHLH071 and bHLH093 also play roles in regulating guard cell development. (B) Micrographs of wild-type and loss-of-function mutants *speechless*, *mute* and *fama*. (DIC images of epidermes courtesy of D. Bergmann.)

therefore important regulators of the global atmospheric environment [1]. Several negative regulators of stomatal development, including receptor-like proteins [2–5] and a MAP kinase kinase kinase [6], have been shown to inhibit stomatal development, but until recently it has not been known how undifferentiated epidermal cells are selected to enter into the stomatal lineage and induced to undergo terminal differentiation into a guard cell pair. The discovery that the regulation of water-use efficiency in plants occurs, in part, by changes in stomatal density [5] underlines the importance of understanding these mechanisms.

Three recent papers [7–9] have shed light on this process by identifying putative transcription factors as 'master regulators' of sequential steps in stomatal differentiation. SPEECHLESS, MUTE and FAMA are closely related members of a family of *Arabidopsis thaliana* proteins that resemble basic helix-loop-helix domain transcription factors. Each of these three proteins has a distinct expression pattern and a specific role in coordinating stomatal determination and differentiation.

Stomatal development in *Arabidopsis* is characterised by a series of cell divisions [10,11] (Figure 1A). Entry into the stomatal lineage is initiated by the division of an undifferentiated post-embryonic epidermal cell to give two unequally sized daughter cells. The smaller cell resulting from this asymmetric division, known as a meristemoid, has transient stem-cell-like properties. The meristemoid undergoes several 'amplifying' asymmetric divisions, which regenerate a meristemoid and produce further sister cells, before differentiating into an oval-shaped guard mother cell. The guard mother cell undergoes a single symmetric division to produce the guard cell pair, and the stomatal pore forms between these cells. The sister cells develop into epidermal cells, but may divide again asymmetrically to produce additional satellite meristemoids.

SPEECHLESS is required for the first asymmetric 'entry' division into the stomatal lineage [7,8]. In early development, *SPEECHLESS* expression becomes restricted to a subset of cells within the developing epidermal layer. These *SPEECHLESS*-expressing cells, which are visually undifferentiated, appear to become competent to divide asymmetrically and initiate a stomatal lineage (Figure 1A). Mutant plants with no expression of the *SPEECHLESS* gene are unable to produce any meristemoids, guard mother cells or stomata (Figure 1B); they grow slowly and do not reach maturity. Plants homozygous for less severe *SPEECHLESS* alleles produce a reduced number of stomata, with a reduced number of sister cells, suggesting that SPEECHLESS is also involved in promoting the asymmetric 'amplifying' divisions of meristemoids. SPEECHLESS does not, however, appear to have roles in promoting asymmetric divisions outside the stomatal lineage, such as those involved in embryogenesis or root development.

MUTE, the gene for which is expressed at a high level in meristemoids, especially after several amplification divisions, and at lower levels in guard mother cells, is required for termination of the stem-cell-like asymmetric division activity and promotion of differentiation [7,8] (Figure 1A). Loss-of-function *mute* plants

produce meristemoids but have no stomata; *mute* meristemoids undergo excessive asymmetric 'amplification' divisions, and become surrounded by a 'rosette' of sister cells, but they cannot progress further through the stomatal lineage to differentiate into guard mother cells (Figure 1B). In contrast, ectopic overexpression of *MUTE* results in an epidermis that consists entirely of stomata.

The FAMA transcription factor, which regulates the later stages of stomatal development, was identified as the product of a gene that is expressed more highly in plants with an excess number of stomata [6], and has been shown to act as a transcriptional activator [9]. FAMA is specifically expressed in the guard mother cell and in immature guard cells, and is required for differentiation into stomata (Figure 1A). Mutant plants with negligible *FAMA* expression have no normal stomata. In their place, *fama* plants produce long clusters of small cells which express genes associated with the stomatal lineage [9] (Figure 1B). These clusters appear to be the result of repeated symmetric divisions of the guard mother cell, producing immature guard cells which are unable to terminally differentiate. Thus, FAMA is necessary to prevent further mitotic divisions of the guard mother cell after the single division that normally gives rises to a guard cell pair, and also to promote guard cell fate. Ectopic over-expression of *FAMA* results in an epidermis consisting entirely of unpaired guard cells, and can even promote the differentiation of guard cells in inappropriate places such as within the mesophyll layer or root epidermis.

FOUR LIPS (FLP) and MYB88, two putative R2R3-type MYB transcription factors, have, like FAMA, been shown to be involved in the differentiation of guard cells from guard mother cells [2,12]. Cell-fate determination of other plant cell types, such as root hair differentiation in the root epidermis, is regulated by a transcription factor complex including a basic helix-loop-helix domain protein and an R2R3-type MYB [13,14]. However, FAMA does not possess the domain which promotes basic helix-loop-helix domain protein/MYB interactions, and no interactions between FLP or MYB88 and FAMA could be detected by various techniques, suggesting that FAMA acts independently of these two MYBs [9].

Unexpectedly, it was found that two other putative basic helix-loop-helix domain transcription factors, bHLH071 and bHLH093, which exhibit significant sequence homology with FAMA, interact with FAMA [9]. Overexpression of the genes for either of these bHLH interactors also causes a weak clustered stomata phenotype, suggesting that they too are involved in promoting stomatal fate. Unlike *FAMA*, expression of which is restricted to guard mother cells and young guard cells, the *bHLH071* and *bHLH093* genes are expressed throughout the plant. It therefore appears that, rather than acting in concert with MYB transcription factors, FAMA may function via interaction with other basic helix-loop-helix domain proteins.

This scenario has parallels with the regulation of cell fate in animal systems, where neuron and muscle cell development involve a similar switch from cell division to terminal differentiation [15,16]. Both of these animal cell differentiation processes are controlled by families of tissue-specific transcription factors which are known as master regulators of cell fate. These master regulators, like SPEECHLESS, MUTE and FAMA, have restricted expression patterns and, like FAMA, interact with other ubiquitously expressed basic helix-loop-helix domain proteins. It appears that, in the case of stomata at least, plants may have adopted a similar mechanism to animals for the differentiation of cells.

These are an extremely significant set of results as they help to unlock the mechanism responsible for the control of stomatal development. To paraphrase Neil Armstrong 'three small steps in developmental biology but three giant steps for stomatal development'.

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